

WHAT IS CLAIMED IS:

1. An attenuated tumor-targeted bacteria comprising ~~one or more nucleic acid~~ molecules encoding one or more primary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

2. An attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

Sub 5.7 3. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein at least one of the primary effector molecules is a TNF family member.

4. The attenuated tumor-targeted bacteria of claim 3, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ -related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.

Sub 5.7 5. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein at least one of the primary effector molecules is an anti-angiogenic factor.

6. The attenuated tumor-targeted bacteria of claim 5, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

Sub 5.7 33 7. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein at least one of the primary effector molecules is a bacteriocin family member with the proviso said bacteriocin is not BRP.

8. The attenuated tumor-targeted bacteria of claim 7, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b, ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A, Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin, or microcin M15.

5 ~~Sub 7~~ 9. The attenuated tumor targeted bacteria of claim 1 or 2, wherein the primary effector molecule is a tumor inhibitory enzyme.

10 10. The attenuated tumor targeted bacteria of claim 9, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.

~~Sub 7~~ 11. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein the primary effector molecule is hemolysin, verotoxin, CNF1, CNF2, or PMT.

15 12. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein the primary effector molecule is derived from an animal, plant, bacteria, or virus.

20 13. The attenuated tumor-targeted bacteria of claim 2, wherein the secondary effector molecule is an immunomodulating agent, an anti-tumor protein, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.

14. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein the attenuated tumor-targeted bacteria is *Salmonella*.

25 15. The attenuated tumor-targeted bacteria of claim 1, wherein the attenuated tumor-targeted bacteria further comprises an enhanced release system.

~~Sub 7~~ 16. The attenuated tumor-targeted bacteria of claim 2, wherein the secondary effector molecule is a bacteriocin release factor (BRP).

30 17. An attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion protein comprises a signal sequence and an effector molecule.

35 18. An attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters,

wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion protein comprises a ferry peptide and an effector molecule.

19. The attenuated tumor-targeted bacteria of claim 18, wherein the fusion protein further comprises a signal sequence.

5 20. The attenuated tumor-targeted bacteria of claim 17 or 19, wherein the signal sequence is an OmpA-like protein.

10 21. The attenuated tumor-targeted bacteria of claim 18 or 19, wherein the ferry peptide is derived from the HIV TAT protein, the antennapedia homeodomain (penetraxin), Kaposi fibroblast growth factor (FGF) membrane-translocating sequence (MTS), herpes simplex virus VP22, hexahistidine, hexalysine, or hexaarginine.

15 22. The attenuated tumor-targeted bacteria of claim 17, 18 or 19, wherein the effector molecule is a primary or secondary effector molecule.

23. The attenuated tumor-targeted bacteria of claim 17, 18 or 19, wherein the attenuated tumor-targeted bacteria further comprises one or more nucleic acid molecules encoding one or more effector molecules operably linked to one or more promoters.

20 24. The attenuated tumor-targeted bacteria of claim 23, wherein the effector molecule is a primary or secondary effector molecule.

25 25. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

30 26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

35 <sup>Sub 37</sup> 27. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecules is a TNF family member.

28. The pharmaceutical composition of claim 27, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ -related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.

Sub 88 29. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecules is an anti-angiogenic factor.

10 30. The pharmaceutical composition of claim 29, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal-proteolytic-fragments of fibronectin, a uPA-receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of Thrombospondin I, 15 the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

20 Sub 89 31. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecules is a bacteriocin family member with the proviso said bacteriocin is not BRP.

25 32. The pharmaceutical composition of claim 31, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A, Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin or microcin M15.

Sub 10 30 33. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecule is a tumor inhibitory enzyme.

34. The pharmaceutical composition of claim 33, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.

Sub 11 35 35. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecule is hemolysin, verotoxin, CNF1, CNF2, or PMT.

36. The pharmaceutical composition of claim 25 or 26, wherein the primary effector molecule is derived from an animal, plant, bacteria, or virus.

37. The pharmaceutical composition of claim 26, wherein the secondary effector molecule is an immunomodulating agent, an anti-tumor protein, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.

38. The pharmaceutical composition of claim 25 or 26, wherein the attenuated tumor-targeted bacteria is *Salmonella*.

39. The pharmaceutical composition of claim 25, wherein the attenuated tumor-targeted bacteria further comprises an enhanced release system.

40. The pharmaceutical composition of claim 26, wherein the secondary effector molecule is a bacteriocin release factor.

41. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion protein comprises a signal sequence and an effector molecule.

42. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion protein comprises a ferry peptide and an effector molecule.

43. The pharmaceutical composition of claim 42, wherein the fusion protein further comprises a signal sequence.

44. The pharmaceutical composition of claim 41 or 43, wherein the signal sequence is an OmpA-like protein.

45. The pharmaceutical composition of claim 42 or 43, wherein the ferry peptide is derived from the HIV TAT protein, the antennapedia homeodomain (penetraxin), Kaposi fibroblast growth factor (FGF) membrane-translocating sequence (MTS), herpes simplex virus VP22, hexahistadine, hexalysine, or hexaarginine.

46. The pharmaceutical composition of claim 41, 42 or 43, wherein the effector molecule is a primary or secondary effector molecule.

47. The pharmaceutical composition of claim 41, 42 or 43, wherein the attenuated tumor-targeted bacteria further comprises one or more nucleic acid molecules encoding one or more effector molecules operably linked to one or more promoters.

48. A method for delivering one or more primary effector molecules for the treatment of a solid tumor cancer to a subject in need of such treatment, comprising administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

49. A method for delivering one or more primary effector molecules and one or more secondary effector molecules for the treatment of a solid tumor cancer to a subject in need of such treatment, comprising administering a pharmaceutical composition a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

50. The method of claim 48 or 49, wherein at least one of the primary effector molecules is a TNF family member.

51. The method of claim 50, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ - related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.

52. The method of claim 48 or 49, wherein at least one of the primary effector molecules is an anti-angiogenic factor.

53. The method of claim 52, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-

angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

5 ~~Sub 54~~ 54. The method of claim 48 or 49, wherein at least one of the primary effector molecules is bacteriocin family member with the proviso said bacteriocin is not BRP.

10 55. The method of claim 54, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b, ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A, Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin or microcin M15.

15 ~~Sub 56~~ 56. The method of claim 48 or 49, wherein at least one of the primary effector molecules is a tumor inhibitory enzyme.

57. The method of claim 56, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.

20 ~~Sub 58~~ 58. The method of claim 48 or 49, wherein at least one of the primary effector molecules is hemolysin, verotoxin, CNF1, CNF2 or PMT.

59. The method of claim 48 or 49, wherein at least one of the primary effector molecules are derived from an animal, plant, bacteria, or virus.

25 ~~Sub 60~~ 60. The method of claim 49, wherein at least one of the ~~secondary effector~~ molecule is an anti-tumor protein, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.

30 ~~Sub 61~~ 61. The method of claim 48 or 49, wherein the attenuated tumor-targeted bacteria is *Salmonella*.

62. The method of claim 48, wherein the attenuated tumor-targeted bacteria further comprises an enhanced release system.

35 63. The method of claim 49, wherein the secondary effector molecule is a bacteriocin release factor.

5 64. A method for delivering one or more fusion proteins for the treatment of a solid tumor cancer to a subject in need of such treatment, comprising administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion proteins comprise a signal sequence and an effector molecule.

10 65. A method for delivering one or more fusion proteins for the treatment of a solid tumor cancer to a subject in need of such treatment, comprising administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion proteins comprise a ferry peptide and an effector molecule.

15 66. The method of claim 65, wherein the fusion protein further comprises a signal sequence.

20 67. The method of claim 64 or 66, wherein the signal sequence is an OmpA-like protein.

25 68. The method of claim 65 or 66, wherein the ferry peptide is derived from the HIV TAT protein, the antennapedia homeodomain (penetraxin), Kaposi fibroblast growth factor (FGF) membrane-translocating sequence (MTS), herpes simplex virus VP22, hexahistidine, hexalysine, or hexaarginine.

69. The method of claim 64, 65 or 66, wherein the effector molecule is a primary or secondary effector molecule.

30 70. The method of claim 64, 65 or 66, wherein the attenuated tumor-targeted bacteria further comprises one or more nucleic acid molecules encoding one or more effector molecules operably linked to one or more promoters.

35 71. A method of treating a solid tumor cancer in an animal, comprising administering one or more chemotherapeutic agents and a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector

molecules operably linked to one or more promoters, wherein said ~~attenuated tumor-targeted~~ bacteria is a ~~facultative aerobe or facultative anaerobe~~.

72. A method of treating a solid tumor cancer in an animal, comprising administering one or more chemotherapeutic agents and a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecule operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

10 ~~Sub 520~~ 73. The method of claim 71 or 72, wherein at least one of the primary effector molecules is a TNF family-member.

74. The method of claim 73, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ -related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.

20 ~~Sub 521~~ 75. The method of claim 71 or 72, wherein at least one of the primary effector molecules is an anti-angiogenic factor.

76. The method of claim 75, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

35 ~~Sub 522~~ 77. The method of claim 71 or 72, wherein at least one of the primary effector molecules is a bacteriocin family member with the proviso said bacteriocin is not BRP.

78. The method of claim 77, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A,

Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin, or microcin M15.

~~Sub 79. The method of claim 71 or 72, wherein at least one of the primary effector molecule is a tumor inhibitory enzyme.~~

5           80. The method of claim 79, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.

~~Sub 81. The method of claim 71 or 72, wherein at least one of the primary effector molecule is hemolysin, verotoxin, CNF1, CNF2, or PMT.~~

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~~82. The method of claim 71 or 72, wherein the primary effector molecule is derived from an animal, plant, bacteria, or virus.~~

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83. The method of claim 82, wherein the secondary effector molecule is an immunomodulating agent, an anti-tumor protein, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.

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84. The method of claim 71 or 72, wherein the attenuated tumor-targeted bacteria is *Salmonella*.

~~85. The method of claim 71, wherein the attenuated tumor-targeted bacteria further comprises an enhanced release system.~~

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86. The method of claim 72, wherein the secondary effector molecule is a bacteriocin release factor.

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87. A method of treating a solid tumor cancer in an animal, comprising administering one or more chemotherapeutic agents and a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion protein comprises a signal sequence and an effector molecule.

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88. A method of treating a solid tumor cancer in an animal, comprising administering one or more chemotherapeutic agents and a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria

comprising one or more nucleic acid molecules encoding one or more fusion proteins operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe and said fusion protein comprises a ferry peptide and an effector molecule.

5        89.    The method of claim 88, wherein said fusion protein further comprises a signal sequence.

90.    The method of claim 87 or 89, wherein the signal sequence is an OmpA-like protein.

10       91.    The method of claim 88 or 89, wherein the ferry peptide is derived from the HIV TAT protein, the antennapedia-homeodomain (penetraxin), Kaposi fibroblast growth factor (FGF) membrane-translocating sequence (MTS), herpes simplex virus VP22, hexahistadine, hexalysine, or hexaarginine.

15       92.    The method of claim 87, 88 or 89, wherein the effector molecule is a primary or secondary effector molecule.

20       93.    The method of claim 87, 88 or 89, wherein the attenuated tumor-targeted bacteria further comprises one or more nucleic acid molecules encoding one or more effector molecules operably linked to one or more promoters.

25       94.    A method of treating a solid tumor cancer in an animal, comprising administering one or more chemotherapeutic agents and a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria.

95.    A fusion protein comprising an OmpA-like protein and an effector molecule.

30       96.    A fusion protein comprising a signal sequence, a ferry peptide and an effector molecule.

97.    The fusion protein of claim 96, wherein the signal sequence is an OmpA-like protein.

35       98.    The fusion protein of claim 96, wherein the ferry peptide is derived from the HIV TAT protein, the antennapedia homeodomain (penetraxin), Kaposi fibroblast growth factor (FGF) membrane-translocating sequence (MTS), herpes simplex virus VP22,

hexahistadine, hexalysine, or hexaarginine.

99. The fusion protein of claim 95 or 96, wherein the effector molecule is a primary or secondary effector molecule.

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